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Sexually Dimorphic Cubomedusa *Carybdea sivickisi* (Cnidaria: Cubozoa) in Seto, Wakayama, Japan

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Abstract The small but distinctive cubomedusa *Carybdea sivickisi* has been reported from a range of tropical, subtropical, and mild temperate localities in the Pacific. In Japan, it has only definitively been documented in the subtropical region of Okinawa. However, in 1970 Uchida noted that three specimens from Seto, Wakayama, which he had referred to as *Tamoya alata* in 1929, were really *C. sivickisi*. The presence of this species as far north as Wakayama was questionable because of the confusion about the identity of the specimens and the fact that *C. sivickisi* had never been observed subsequently in this region. In August 2006, we discovered a population of this tiny species nearby the Seto Marine Biological Laboratory in Shirahama, Wakayama. In this paper we comment and clarify the geographical range, developmental morphology, and sexual behaviour of this species.

Key words: courtship, spermatophore, nocturnal, reproduction, geographic ranges, medusae

Introduction

Carybdea sivickisi Stiasny, 1926 is a relatively small, sexually dimorphic cubomedusa, up to 14 mm in diameter, with four distinctive adhesive pads that stick the animal to algal substrates during the day (Hartwick, 1991; Lewis and Long, 2005). Mature females display leaf-life gonads (2 per quadrant), while male gonads are in the form of two orange-tinted sperm-filled hemigonads per quadrant. *C. sivickisi* has been sporadically reported from diverse tropical, subtropical, and mild temperate localities in the Pacific Ocean, with one exception in the Indian Ocean (Table 1). In Japan, *C. sivickisi* has been well-documented in Okinawa (see Lewis and Long, 2005). There has also been a single report of the species in Wakayama (Uchida, 1970). The latter appears in the published revision of Japanese Cubozoa (Uchida, 1970), in which Uchida stated that specimens from Seto, Wakayama, which he described under a different name in an earlier publication (Uchida, 1929), were most likely *C. sivickisi*. Findings in the present study corroborate Uchida's speculation that *C. sivickisi* is present in Seto waters. Given the cubomedusa's nocturnal and cryptic tendencies, however, it has probably gone undetected.

During the summer of 2006, in the course of collecting diverse cnidarians for the Cnidarian Tree of Life project (http://cnidarian.info), we encountered a single specimen of *C. sivickisi* during an early morning (5:30 AM) plankton tow. This was surprising due to nocturnal nature of *C. sivickisi*, and it prompted us to search a nearby harbour locality at night, where we were able to sample numerous additional animals of both sexes. Using these animals, we were able to replicate the courtship and

Table 1. Reported sightings of *C. sivickisi* since 1922. Not exhaustive.

Locality Name	**Sampling Date**	**Publication/Source**
Japan: Seto, Wakayama	Aug., prior to 1929 Aug, 2006	Uchida, 1929 This Study
Japan: Okinawa, Motobu Shinko and Okinawa Prefectural Sea Farming Center (Northwest); Nakagusuku Bay (Southeast); Ginowan Marina (Southwest) North of Miyako-jima, Dana St. 3723	Apr. – Aug., 1994-1997 Jan., 1929	Lewis and Long, 2005 ZMUC
USA: Hawaii, O'ahu: Mamala Bay (South), Sans Souci Beach, Waikiki and outside Yokohama Bay (West); Maui: Ma'alaea Boat Harbor	Jul., 1996 & Mar., 1998 Jul., 2005 & Feb., 2006	Matsumoto et al., 2002 Crow et al., 2006
The Philippines: Puerto Galera, Mindoro	Apr. – May, 1924	Stiansy, 1926
Guam	unknown	Gershwin, 2003
Vietnam: Nhathrang Bay	Oct., 1959	ZMUC
Thailand: The Gulf of Siam (Sund Koh Chang)	Jan., 1900	Stiansy, 1922
Indonesia: West of Sumatra, Dana St. 3828	Sep., 1929	ZMUC
Australia: Magnetic Island, Townsville	Oct. – Nov., 1982 & 1989 Oct., 1993	Hartwick, 1991 NMNH 97389
New Zealand: Island Bay, Wellington	year round, primarily between Nov. & Feb., 1973, 1977, 1980, & 1984	Hoverd, 1985

sexual behaviours documented by Lewis and Long (2005). This is just the second time that courtship resulting in copulation has ever been reported of any cubozoan (or cnidarian). Our new observations allow us to briefly clarify the development, morphology, and sexual behaviour of *C. sivickisi*.

Material and Methods

The first sample of *Carybdea sivickisi* was an immature female medusa (bell diameter 2.5mm) collected by a skin diver doing a plankton tow just northwest of the Seto Marine Biological Laboratory at 05:30 on 11 August 2006. No *C. sivickisi* medusae were recovered in subsequent plankton tows (both daytime and night time) in that area. However, *C. sivickisi* medusae were collected at the Seto fishing port of Shirahama, Wakayama between 14 and 18 August 2006. Individuals were attracted using a light between 20:00 and 22:00. A total of 25 medusae - 12 females, 10 males and three juveniles - were collected with a plankton net or hand-held scoop. At this same locality, *Carybdea mora* Kishinouye, 1910 (often synonymised with *Carybdea rastonii* Haacke, 1886, but see Gershwin, 2006), *Mastigias papua* (Lesson, 1830), *Dipurena ophiogaster* Haeckel, 1879, and *Liriope tetraphylla* (Chamisso and Eysenhardt, 1821) were collected. Medusae of *C. mora* had not been reported from the Shirahama region for several years, but medusae were abundant at the water surface during the day and feeding at night. After being measured and used in the experiments described below, all collected *C. sivickisi* medusae were anaesthetized in 7% $MgCl_2$ and preserved in 99% EtOH or 5% formalin solution for future histological studies and DNA analysis.

C. sivickisi medusae were numerous during the time of sampling. The majority appeared to be less than 3 mm in diameter, and larger (adult) specimens were preferentially targeted for collecting. The average diameter of all medusae collected from the Seto fishing port was 2.8 mm (1.0 - 5.5 mm in range, n=25). The average bell diameters for the three juveniles, ten immature females, two mature females (with velar spots), three immature males, and seven mature males were 1.3, 3.4, 3.5, 4.8, 5.0 mm, respectively. Male and female individuals were separated upon collection, kept separately in glass jars (500 ml - 1000 ml), and fed *Artemia* nauplii. Sea water was changed daily.

Two courtship experiments similar to those of Lewis and Long (2005) were conducted in the lab, one each on the evenings of 15 and 17 August 2006. Courtship behaviour occurred within a few minutes and medusae were kept in the bottles until interaction between males and females ceased (80 min on 15 August, and 60 min on 17 August). Following spermatophore transfer, females were stored individually and monitored for embryo strand production. The resulting embryo strand was reared (see Results).

Results

Development and Morphology

Among the individuals collected in Shirahama, three showed no sign of gonad development and could not be sexed (smallest: 1.0 mm; average diameter 1.3 mm). In these juveniles, a pair of orange subgastric sacs (typical of both males and females) was faintly visible below each of the gastric phacellae continuous to the gastric ostium (Fig. 1A). Sexual dimorphism was apparent in individuals larger than 2.5 mm in diameter. Specifically, females display two leaf-life gonads per quadrant; whereas male gonads are in the form of two orange-tinted sperm-filled hemigonads per quadrant (compare Fig. 1C with Fig. 2B). Individuals with diameters greater than 4.5 mm (males n=8; females n=3) had mature gonads, with the exception of one male and one female bearing immature gonads. Otherwise, females (n=11) from 2.5 mm to approximately 4.0 mm in diameter had immature gonads.

Gonads were noticeable in males of bell diameter greater than 3.0 mm (n=10). They were marked by four pairs of small flat hemispheres at each interradius. Observations on individuals from Okinawa (Lewis, pers. obs.) revealed that as these hemispheres enlarge, a small amount of orange pigment appears within each hemigonad (Fig. 1B). In this study, in all but one male with a bell diameter greater than 4.0 mm, these orange patches had developed into a pair of flattened hemigonads, which were pinched in at the perradius. In larger males, the paired hemigonads contained an orange

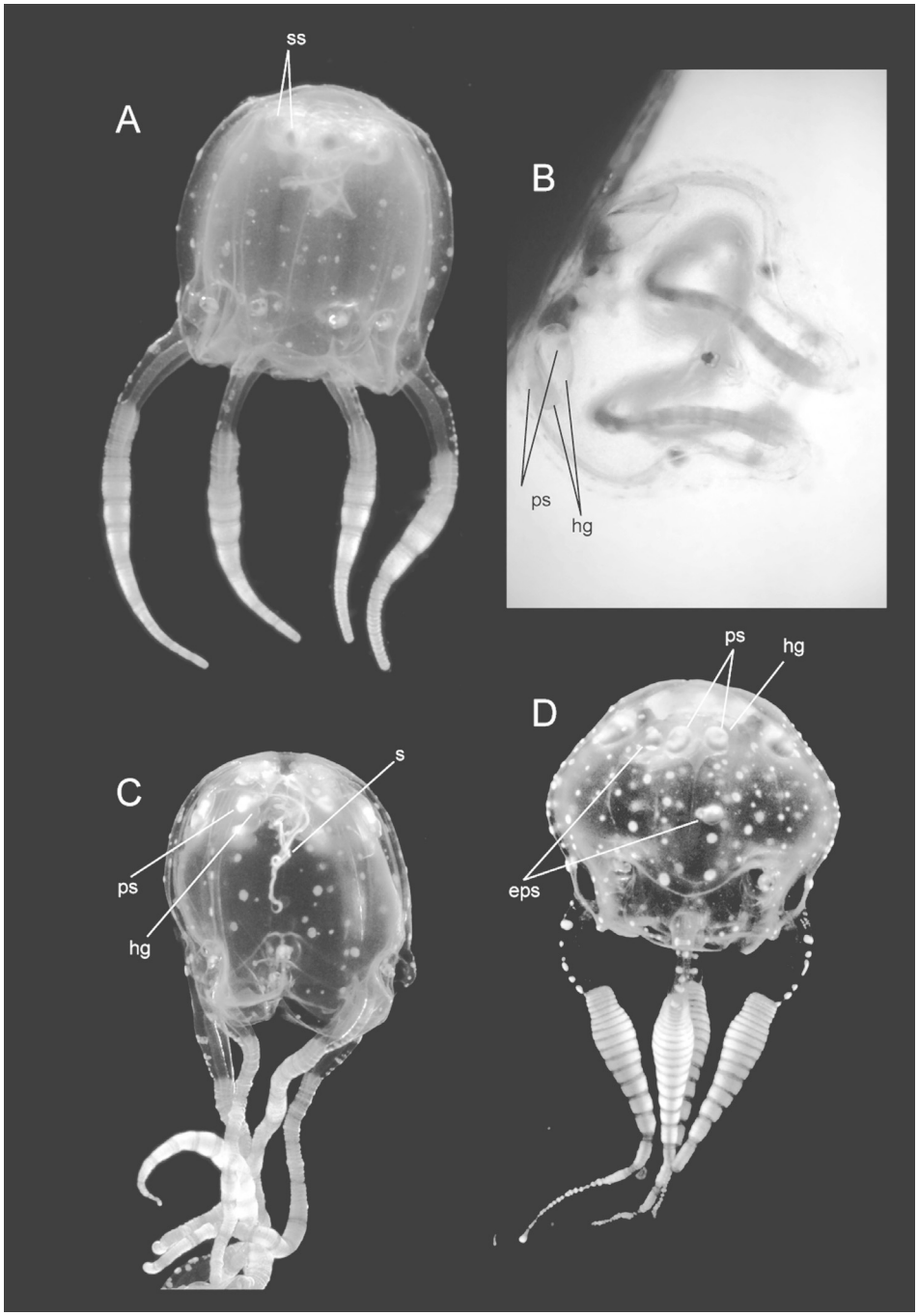

Fig. 1. A-D. Juvenile and male medusae of *Carybdea sivickisi*. A. juvenile of undetermined sex. B. immature male, roughly 3 mm in diameter, adhering to substrate with apical pads. C. young adult male, roughly 5 mm in diameter, releasing spermatophore during copulation. D. young adult male, roughly 4.5 mm in diameter, with two extra pigmented structures. Abbreviations: eps - extra pigmented structure, hg - hemigonad, ps - pigment spot/pigmented structure, s - spermatophore, ss - subgastric sacs.

pigmented structure filled with sperm (see Hartwick, 1991; Fig. 1C). It should also be noted that one mature male (approximately 4.5 mm in diameter) had two extra orange pigmented structures that appeared to have developed in the gastric ostium: one mid-way down in the center of the bell and another which had developed on the perradial part of one of the male's hemigonads (Fig. 1D). Similar "extra" gonadal material was occasionally observed in male *C. sivickisi* specimens from Okinawa (Lewis, pers. obs.). Their derivation and functionality are unknown.

Females with bell diameters greater than 2.5 mm (n=12) bore pairs of gonads (gastric pockets) at each interradius. Similar to Okinawa individuals, the gonads began as narrow structures (<1 mm in length) (Lewis, pers. obs.) (Fig. 2A) and elongated downward into sheets of pale tissue as the medusa reaches a bell diameter of 4.5 mm (n=3) (Fig. 2B). Velar spots - pigmented terminal regions of structures that branch off from the velar canals (4 per quadrant) - were conspicuous in one mature female (diameter 5 mm) and pale in the other (diameter 4.5 mm). The female with conspicuous velar spots had cloudy gastric pockets, which under the dissecting microscope appeared grainy (Fig. 2C). Based on previous observations on Okinawa *C. sivickisi* (Lewis and Long, 2005), the medusa was likely carrying embryos from a fertilization prior to collection.

In both males and females, a pair of subgastric sacs exist below each of the gastric phacellae (2 per quadrant), continuous to the gastric ostium. These sacs have dark orange pigmentation and appear to function as sperm-storage organs: spermathecae in females and seminal vesicles in males (Hartwick, 1991; Lewis and Long, 2005; observations detailed below).

Sexual Behaviour

During the two *in vitro* courtship experiments, copulation was observed four and three times, respectively. Within several minutes the male attached a tentacle to one of the females' tentacles (Fig. 2D). The male gained control pulling her around. Contracting his tentacles he brought the oral openings of the male and female together, all the while producing a spermatophore (Fig. 1C). With the oral openings in contact, the male transferred the spermatophore to the female (Fig. 2D). Spermatophores appeared lighter in colour and thinner than those produced by larger males (of diameter 7-10 mm in range) in similar courtship experiments in Okinawa (Lewis and Long, 2005). Nevertheless, the average number of spermatophore transfers per trial (n=4; 1-8 in range) and the courtship and sperm-transfer dynamics were similar to that observed in Okinawa.

In the first courtship trial (15 August), embryo strand release occurred 24 hours after the female ingested the first spermatophore (48 hours after collection). In the subsequent courtship trial (17 August), shortly after ingesting the third spermatophore (thus prior to releasing an embryo strand), the female was anaesthetized in 7% $MgCl_2$ and fixed in a 5% formalin solution for future histological studies. Given that the female in the first trial had cloudy gastric pockets when collected, the resulting embryo strand was most likely due to copulation in the field, prior to the time of collection, and not a result of the copulations that took place during the courtship experiments. In Okinawa *C. sivickisi*, the gestation period for embryo strands resulting from *in vitro* fertilizations was 55 h (44-66 h in range; n=5), whereas embryo strands recovered from females apparently fertilized before their capture were exuded an average of 39 h after capture (22-57 h in range; n=22) (Lewis and Long, 2005).

Embryo strands resulted in the release of planulae that subsequently settled and developed into multi-tentacular polyps in glass dishes. As in other studies (Hartwick, 1991; Lewis and Long, 2005), the *C. sivickisi* polyps died within a month of settlement on the substrate without metamorphosing into juvenile medusae.

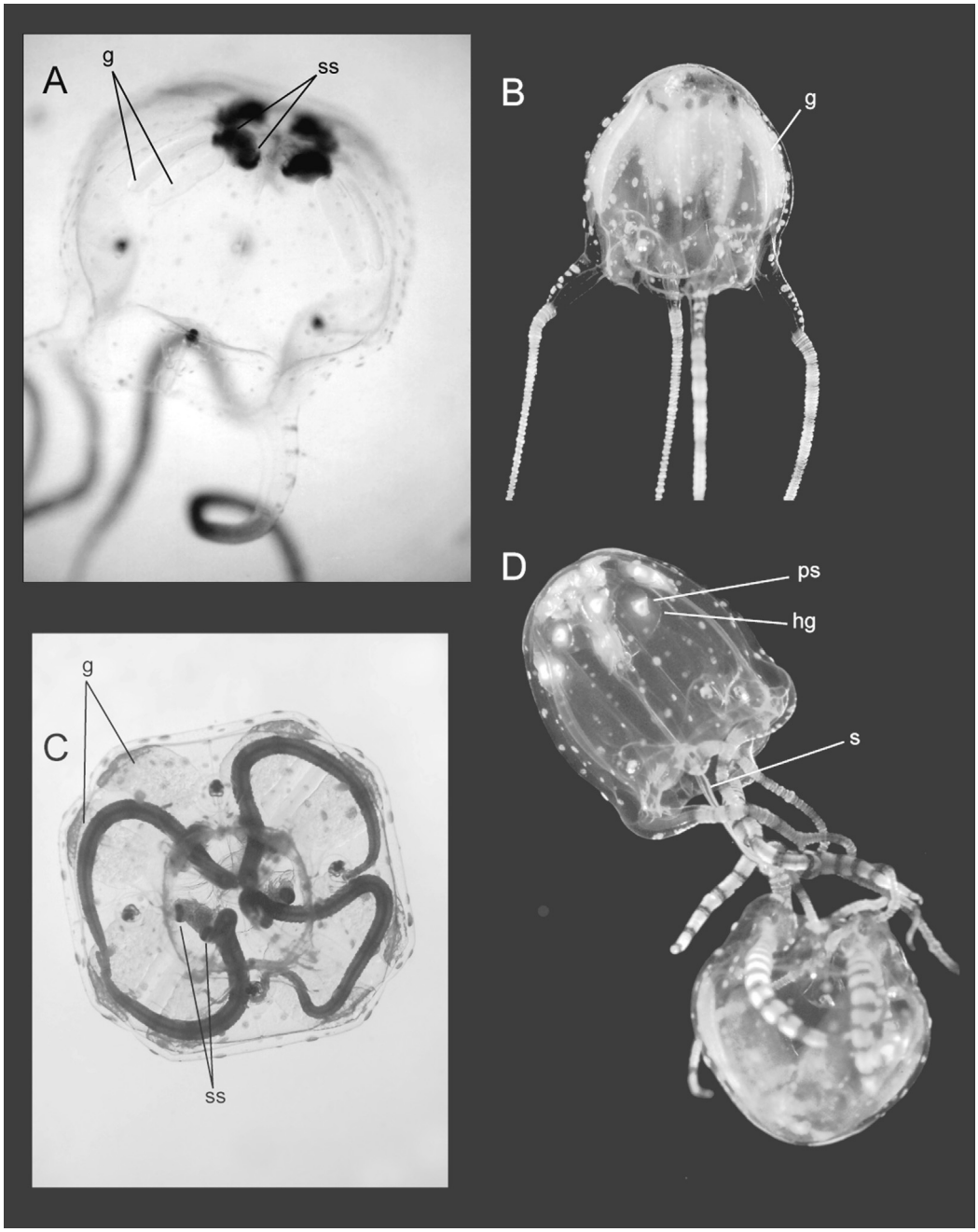

Fig. 2. A-D. Female medusae and copulation of *Carybdea sivickisi*. A. immature female, roughly 3 mm in diameter. B. young adult female, roughly 4.5 mm in diameter, with cloudy gonads potentially indicating prior insemination. C. same female as in 2B, oral view with tentacles inside its subumbrella. D. young adult male (above) transferring spermatophore to young adult female (below). Abbreviations: g - gonads, hg - hemigonad, ps - pigment spot/pigmented structure, s - spermatophore, ss - subgastric sacs.

Discussion

Based on comparison of Uchida's (1929, 1970) descriptions and the observations made here and in Okinawa (Lewis and Long, 2005), it is clear that Uchida (1929) had in fact documented the occurrence of *Carybdea sivickisi* in Seto, Wakayama, Japan in his description of what he called *Tamoya alata*. The timing of maturation of *C. sivickisi* may vary from locality to locality, as the medusa is present in sub-tropical Okinawa waters between April and early August when temperatures range from 23℃ to over 30℃ (Lewis and Long, 2005), while in this study, juveniles and small mature adults were observed together in temperate Seto, Wakayama at the end of August when temperatures approached 30℃. The duration of *C. sivickisi* in the Seto area is unknown.

In both this study and that done by Lewis and Long (2005), *C. sivickisi* females were capable of receiving an average of four spermatophores from a single male, as well as from multiple males. It is difficult, however, to discern how representative these numbers are of the frequency of copulation *in situ* where medusae are not as physically confined, and where only a limited percentage of individuals in the population are sexually mature.

Much remains to be learned about the biology of *C. sivickisi*, but our replication of the sexual experiments in this species suggests that it would make a nice system for further study. One hindrance to supplementary investigation is the failure to be able to rear polyps to the point that they metamorphose into juvenile medusae. It is possible that the polyps require special conditions. Females have been known to release embryo strands on macroalgae when it is present in the rearing tank (Lewis and Long, 2005). Since embryo strands sink when released, perhaps the developing planulae adhere to the stalk or fronds of *Sargassum* or other macroalgae, providing a suitable substrate for polyps until temperature or other environmental conditions are appropriate for release of juvenile medusae. Despite the difficulty of rearing polyps, further investigation of the sexual behaviour of *C. sivickisi* is warranted. Future copulation experiments involving two females with velar spots and one male, two females (one with and one without velar spots) and one male, and just one pair of mature male and female medusae could reveal new findings related to sperm competition in *C. sivickisi*. Ideally, such a study would be carried out after developing a system of microsatellite markers that could be used to test for paternity of planulae derived from embryo strands.

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References

Crow, G.L., Miroz, A., Chan, N. and Lam, K. 2006. Documentation of box jellyfish *Carybdea sivickisi* and *Carybdea rastoni* (Cubozoa: Carybdeidae) at Ma'alea Harbor, Maui. Bishop Museum Occasional Papers no. 88, 2006. Records of the Hawaii Biological Survey for 2004-2005, Part 2, Notes, 55-56.

Gershwin, L. 2003. Scyphozoa and Cubozoa of Guam. Micronesia, 35-36, 156-158.

Gershwin, L. 2005. *Carybdea alata* auct. and *Manokia stiasnyi*, reclassification to a new family with description of a new genus and two new species. Memoirs of the Queensland Museum, 51, 501-523.

Gershwin, L. 2006. Nematocysts of the Cubozoa. Zootaxa, 1232, 1-57.

Hartwick, R.F. 1991. Observations on the anatomy, behaviour, reproduction and life cycle of the cubozoan *Carybdea sivickisi*. Hydrobiologia, 216/217, 171-179.

Hoverd, W.A. 1985. Occurrence of the order Cubomedusae (Cnidaria: Scyphozoa) in New Zealand: collection and laboratory observations of *Carybdea sivickisi*. New Zealand Journal of Zoology, 12, 107-110.

Lewis, C. and Long, T.A.F. 2005. Courtship and reproduction in *Carybdea sivickisi* (Cnidaria: Cubozoa). Marine Biology, 147, 477 - 483.

Matsumoto, G., Crow, G.I., Cornelius, P.F.S. and Carlson, B.A. 2002. Discovery of the cubomedusa *Carybdea sivickisi* (Cubozoa: Carybdeidae) in the Hawaiian Islands. Bishop Museum Occasional Papers no. 69, 2002. Records of the Hawaii Biological Survey for 2000, Part 2, Notes, 44-46.

Stiasny, G. 1922. Papers from Dr. Th. Mortensen's Pacific Expedition 1914-1916. XII. Die Scyphomedusen-Sammlung von Dr. Th. Mortensen nebst anderen Medusen aus dem Zoologischen Museum der Universität in Kopenhagen. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i København, 73, 499-511.

Stiasny, G. 1926. Über einige Scyphomedusen von Puerto Galera, Mindoro (Philippinen). Zoologische Mededeelingen, Leiden, 9, 239-248.

Uchida, T. 1929. Studies on the Stauromedusae and Cubomedusae with special reference to their metamorphosis. Japanese Journal of Zoology, 2, 103-193, pls. 3-5.

Uchida, T. 1970. Revision of Japanese Cubomedusae. Publications of the Seto Marine Biological Laboratory, 17, 289-297.